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Antimalarial activity of N^6-substituted adenosine derivatives. Part 3

Claudia Herforth,[a] Jochen Wiesner,[b] Philipp Heidler,[c] Silke Sanderbrand,[b] Serge Van Calenbergh,[d] Hassan Jomaa[b] and Andreas Link[c,*]

[a]*Institut für Pharmazie, Abteilung für Pharmazeutische Chemie, Universität Hamburg, Bundesstraße 45, D-20146 Hamburg, Germany*
[b]*Biochemisches Institut, Justus-Liebig Universität Gießen, Friedrichstraße 24, D-35392 Gießen, Germany*
[c]*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany*
[d]*Laboratory for Medicinal Chemstry, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium*

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Abstract—A series of novel 3′-amido-3′-deoxy-N^6-(1-naphthylmethyl)adenosines was synthesized applying a polymer-assisted solution phase (PASP) protocol and was tested for anti-malarial activity versus the Dd2 strain of *Plasmodium falciparum*. Further, this series and 62 adenosine derivatives were analyzed regarding 1-deoxy-D-xylulose 5-phospate (DOXP) reductoisomerase inhibition. Biological evaluations revealed that the investigated 3′,N^6-disubstituted adenosine derivatives displayed moderate but significant activity against the *P. falciparum* parasite in the low-micromolar range. On the molecular level, DOXP reductoisomerase utilizing an adenosyl-containing substrate was identified as a promising metabolic target for ligands of adenosine binding motifs.

1. Introduction

Although the past 5 years have seen a pronounced reawakening of interest in malaria in the richer countries of the world, the burden of malaria is still increasing, especially in sub-Saharan Africa. Particularly drug and insecticide resistance are unsolved problems. Thus there is an urgent need for novel tools in the fight against *Plasmodium falciparum*, the microorganism that causes the most severe form of malaria.[1]

Due to the fact that the parasites' resistance to conventional antimalarial drugs is increasing, the development of drugs attacking novel targets in the metabolism of the malaria pathogen is an imperative. One such family of targets are the enzymes associated with plasmodial isoprenoid synthesis. In mammals and plants, isoprenoid units are generally assembled via the mevalonate pathway, whereas in algae and most bacteria a different pathway is followed. Our investigations are based on the recent discovery that *P. falciparum* utilizes this alternative mevalonate-independent so-called DOXP/MEP (1-deoxy-D-xylulose 5-phospate/2-C-methyl-D-erythitol-4-phosphate) pathway for isoprenoid biosynthesis.[2,3] The DOXP reductoisomerase is a key enzyme in the DOXP/MEP pathway, converting DOXP into MEP using NADPH as hydride donor.[4,5] The inhibition of DOXP reductoisomerase with fosmidomycin (not shown) leads to substantial antimalarial activity in vivo. Because the DOXP/MEP pathway is present in the malaria parasite *P. falciparum*, but is absent in humans, enzymes of the DOXP/MEP pathway are clean and valid targets for antimalarial drug discovery.[3,6]

The development of derivatives of the highly active antibiotic fosmidomycin in order to obtain derivatives with improved pharmacokinetic properties regarding plasma half-live and bioavailability is rewarding, but at the same time highly demanding. Water has to be excluded from the catalytic hydride transfer process resulting in a narrow active site. While active site inhibition clearly holds the key for strong and selective inhibition of DOXP reductoisomerase, it is practical to complement these efforts by approaches focusing on the NADPH binding site of the enzyme.

* Corresponding author. Tel.: +49-6421-282-5900; fax: +49-6421-282-5901; e-mail:

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Generally speaking, adenosine binding motifs are associated with a broad array of therapeutically significant targets in biological systems.[7–11] In the course of ongoing studies establishing structure–activity relationships of N^6-substituted adenosine derivatives as potential inhibitors of the *P. falciparum* pathogen, compound **1** was identified as a lead structure exhibiting antimalarial activity.[12,13] Structural modifications of the lead compound covering the replacement of the 3′-(6-phenylhexanoyl) residue and substitution in N^6-position by a number of acyl residues and 1-naphtalenemethyl, respectively, were performed to address the question how variations in the 3′- and N^6-positions would influence the antimalarial activity and whether these compounds are able to block the NADPH binding site of DOXP reductoisomerase.

Herein, we describe the polymer-assisted synthesis and biological evaluation of modified 'lead-structure' like 3′,N^6-disubstituted adenosine derivatives (Fig. 1). Furthermore, 5′,N^6-disubstituted and N^6-monosubstituted adenosines derived from previous studies, displaying moderate antimalarial activity, were investigated with regard to DOXP reductoisomerase inhibition.[12,13] Conclusions along structure–activity relationships to define general structural features involved in the antimalarial activity are discussed.

2. Chemistry

In the past, we reported on the structure-based design of adenosine analogues as selective micromolar inhibitors of *P. falciparum* (N^6-monosubstituted and 5′,N^6-disubstituted adenosine analogues) and of trypanosomal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (2′,N^6-bisubstituted adenosine analogues).[12–16] Following the same approach, a small series of 3′,N^6-disubtituted adenosines was synthesized and subsequently tested for antimalarial activity. The synthetic approach towards these derivatives required the preparation of a novel nucleoside template (**7**). In order to access a sequence of 3′-amido-3′-deoxy-N^6-(1-naphthylmethyl)-adenosines, a polymer-assisted solution-phase protocol for the chemoselective acylation of the 3′ amino group was employed.

The nucleoside template **7** was obtained by conventional synthesis in solution (Scheme 1). Initially, 3′-azido-3′-deoxyadenosine (**2**) obtained following an elegant procedure described by Robins et al. was simultaneously protected at the 2′- and 5′-positions by treatment with acetic anhydride in pyridine as reported by Ikehara and Takatsuka.[17,18] Crystalline **3** was then subjected to chlorination using tetrachloromethane and isoamylnitrite for the exchange of the 6-amino group for a chlorine atom.[15,19,20] Deprotection with saturated methanolic ammonia and subsequent quantitative aminolysis with (1-naphthylmethyl)amine in 1-propanol yielded compound **6**. The reduction of the azido functionality employing indium/NH_4Cl in ethanol led to a smooth conversion of **6** to the desired novel scaffold **7**. Generally, Pd-catalyzed hydrogenation is used to afford reduction of this type of modified adenosines.[18] Nevertheless, considering the possible loss of the N^6-substituent, Pd-catalyzed hydrogenation seemed not to be suited for our purpose. Only intermediate **3** and compounds **6** and **7** intended for biological evaluation were isolated and characterized by NMR, HPLC and MS.

Diversity fragments were introduced employing a PASP protocol (Scheme 2). Commercially available carboxylic

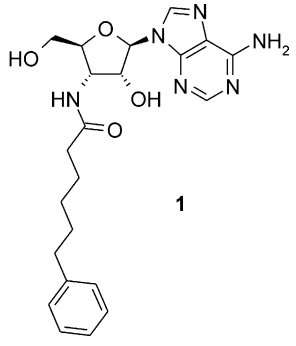
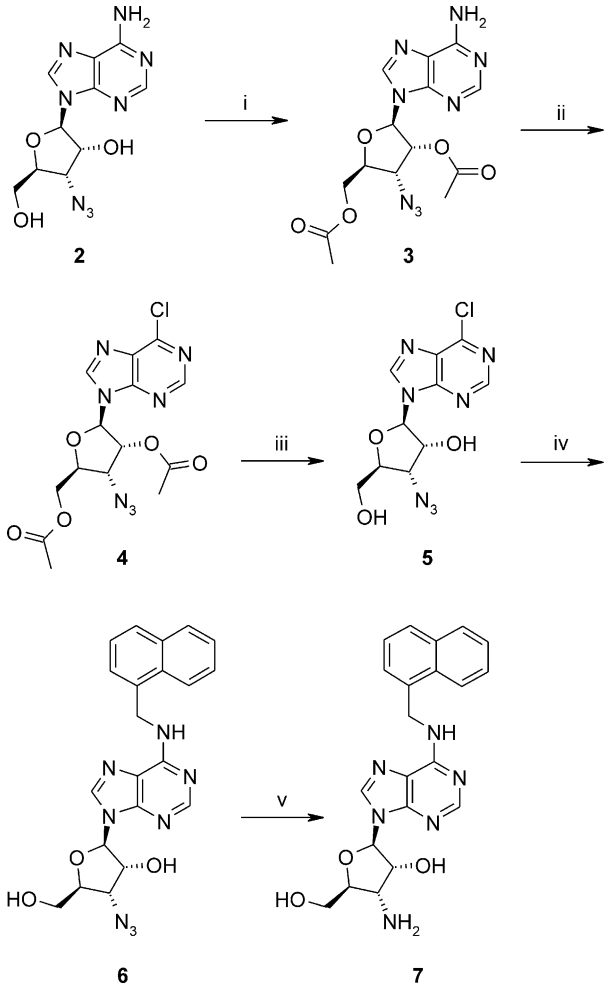


Figure 1. Lead structure (**1**).



Scheme 1. Synthetic sequence yielding 3′-amino-3′-deoxy-N^6-(1-naphthylmethyl)adenosine (**7**): (i) Ac_2O, pyridine abs., ambient temperature, 1 h; (ii) CCl_4, iso-amylnitrite, 60 °C, 24 h; (iii) methanolic ammonia (saturated), 0 °C, 1 h; (iv) 1-naphthylmethylamine, 1-propanol, DIPEA, 50 °C, 4 h; (v) indium/NH_4Cl, ethanol, reflux, 4 h.

acid equivalents were thus attached via in situ anhydride formation to the Kenner safety-catch linker connected to aminomethylated polystyrene. Prior to the chemoselective transfer of the polymer-bound acid to the primary amino group of the nucleoside template **7**, the safety-catch linker had to be activated by *N*-alkylation to form a good leaving group.[21,22] Appropriate activated polymer-bound carboxylic acid equivalents **8a–d** were converted to the corresponding 3′-amido-3′-deoxy-N^6-(1-naphthylmethyl)adenosines **9a–d**, when agitated with a solution of the amino nucleoside **7** at slightly elevated temperatures. The reactions were terminated after TLC analysis indicated consumption of the amino nucleoside. As PASP synthesis generally leads to simple product isolation (because the excess of polymer-bound reagent that is necessary to drive the reaction to completion can be removed by filtration), **9a–d** were obtained in excellent purity and good to excellent yield.

3. Biological evaluation

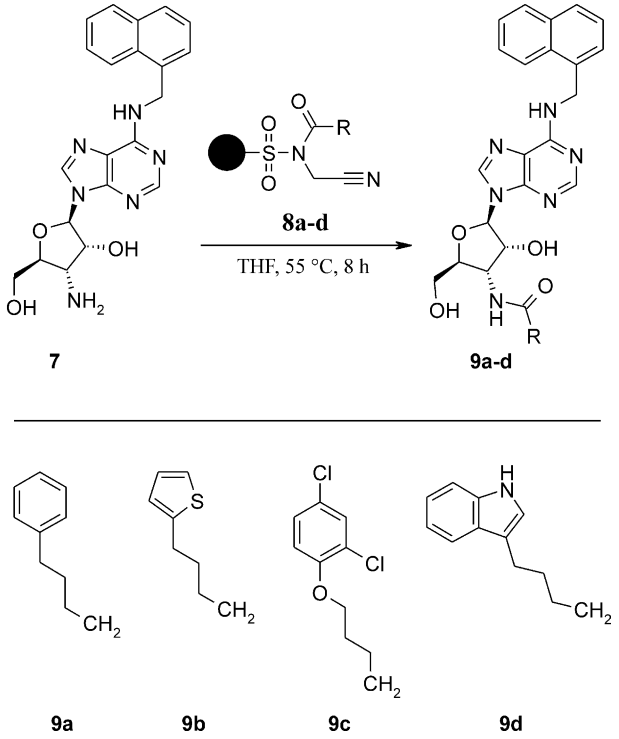
Adenosine analogues **6** and **7** and adenosine derivatives **9a–d** were evaluated for their inhibitory activity against intra-erythrocytic forms of *P. falciparum* using a semi-automated microdilution assay as described.[23–25] The growth of the parasites was monitored through the incorporation of tritium-labelled hypoxanthine. The results obtained are summarized below (Table 1).

For DOXP reductoisomerase inhibition studies, compounds **6**, **7**, and **9a–d** were analyzed. The conversion of DOXP to MEP by the recombinant enzyme was determined in an assay based on NADPH dependency of the reaction.[3,13] For results see Table 1.

In addition, 62 library compounds derived from previous studies were tested for DOXP reductoisomerase inhibition. The synthesis of these 62 adenosine derivatives of types I–III (for main structures see Fig. 2) as well as the inhibitory activity against the *P. falciparum* strain Dd2 has been reported recently.[12,13]

Briefly, 26 type I adenosine derivatives have either been prepared by simple amination of 6-(chloropurin-9-yl)-β-D-1-deoxyribofuranose in solution or by PASP synthesis of N^6-(2-aminoethyl)adenosine and N^6-(3,6-dioxa-8-amino-octyl)adenosine, respectively.[16] Thirty type-II adenosines and six type-III adenosines have been prepared by *N*-selective acylation of 5′-amino-5′-deoxy-N^6-(1-naphthylmethyl)adenosine and 5′-amino-5′-deoxy-N^6-(4-phenyl)benzyladenosine, respectively, following a (modified) PASP protocol comparable to the one described above.

The biological evaluation is summarized in Table 1. As none of the 26 type I adenosine derivatives exhibited an inhibitory activity on DOXP reductoisomerase, only results of type II and III adenosines are listed.



Scheme 2. Polymer-assisted synthesis furnishing 3′-amido-3′-deoxy-N^6-(1-naphthylmethyl)adenosines **9a–d**.

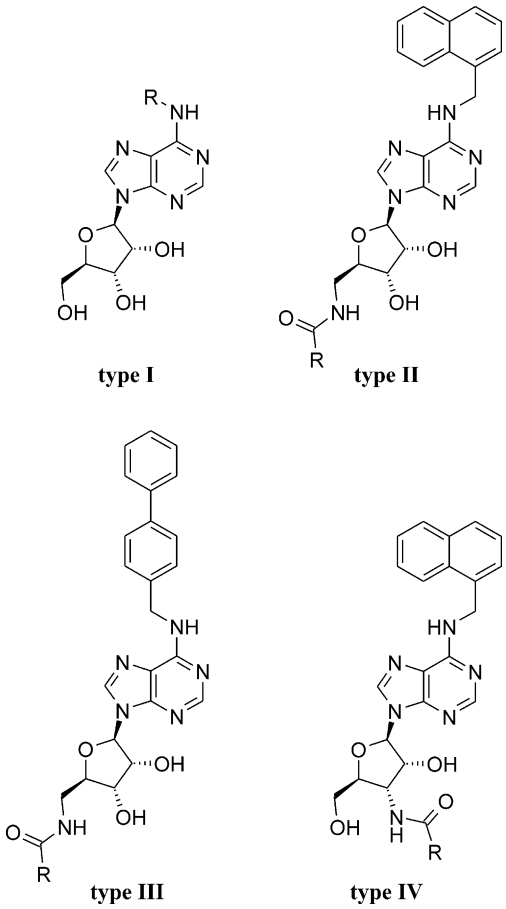


Figure 2. Adenosine derivatives of types I–IV.

Table 1. Antimalarial activity of adenosines **6**, **7**, and type IV derivatives **9a–d** against the *P. falciparum* Dd2 strain and DOXP reductoisomerase inhibition of adenosines **6**, **7**, and type IV derivatives **9a–d**, type II adenosines **10a–c$_1$**, type III adenosines **11a–d**, and three adenosine analogues

Compd	R group in main scaffold	IC_{50} (μM)[a]	DOXP (%)[b]
1	—	3.2	0
6	—	12	0
7	—	11	0
Type IV adenosine derivatives			
9a	(4-Phenylbutanamido)	18	38
9b	[4-(2-Thienyl)butanamido]	18	30
9c	{4-(2,4,-Dichlorophenoxy)butanamido}	15	41
9d	[4-(3-Indolyl)butanamido]	2.8	75
Type II adenosine derivatives			
10a	H	12	0
10b	(Acetamido)	23	0
10c	(Propanamido)	15	0
10d	(Cyclopropanamido)	11	0
10e	[(3-Thienyl)acetamido]	11	0
10f	(2-Phenylbutanamido)	6.2	0
10g	[4-(3-Indolyl)butanamido]	8.5	45
10h	(Diphenylacetamido)	6.2	50
10i	[4-(2,4-Dichlorophenoxy)butanamido]	10	0
10j	{*trans*-3-[(4-Chloro-3-nitro)phenyl]prop-2-enamido}	5.3	52
10k	{*trans*-3-[(3-Nitro-4-propylamino)phenyl]prop-2-enamido}	4.8	33
10l	(3-Methoxybenzamido)	11	0
10m	[(4-Cyclopropylamino-3-nitro)benzamido]	26	0
10n	[4-(1-Methylethyl)amino-3-nitro)benzamido]	4.1	0
10o	[3-Nitro-4-(pyrrolid-1-yl)benzamido]	15	0
10p	[(4-Cyclopentylamino-3-nitro)benzamido]	4.8	47
10q	[3-Nitro-4-(piperid-1-yl)benzamido]	14	0
10r	[4-(Morphol-1-yl)-3-nitro]benzamido	4.8	0
10s	[(4-Dibutylamino-3-nitro)benzamido]	4.2	70
10t	{[4-(2-(2-Methoxyphenyl)ethylamino)-3-nitro]benzamido}	1.3	58
10u	{[4-(Morphol-1-yl)-3-(4-nitrobenzamido)]benzamido}	12	27
10v	{[3-Acetamido-4-(morphol-1-yl)]benzamido}	5.0	0
10w	[3-Acetamido-4-(piperid-1-yl)benzamido]	4.2	24
10x	[3-Acetamido-4-(*N*-benzyl-*N*-ethyl)benzamido]	3.3	65
10y	{4-[2-(2-Methoxyphenyl)ethylamino]-3-propanamido}benzamido	2.7	0
10z	[3-*tert*-Butyloxycarbonamido-4-(morphol-1-yl)]benzamido	3.1	0
10a$_1$	[(3-Acetamido-4-dibut-1-ylamino)benzamido]	3.7	59
10b$_1$	[(4-Dibut-1-ylamino-3-butanamido)benzamido]	3.2	53
10c$_1$	{[4-(1-Methylethyl)amino-3-propanamido]benzamido}	3.2	0
5′-Deoxy-N^6-(1-naphthylmethyl)-5′-pthalimidoadenosine (not shown)		11	0
1-(6-Chloropurin-9-yl)-β-D-1,5-dideoxy-(5-phthalimido)ribofuranose (not shown)		42	42
Type III adenosine derivatives			
11a	H	2.1	41
11b	[4-(3-Indolyl)butanamido]	3.7	48
11c	(3-Methoxyphenylacetamido)	14	0
11d	{[3-Acetamido-4-(morphol-1-yl)]benzamido}	3.2	0
N^6-(1-Biphenylmethyl)-5′-deoxy-5′-phthalimidoadenosine (not shown)		4.3	0

[a] The *P. falciparum* strain Dd2 (Indochina) used in this study is resistant to most commonly used antimalarial drugs. When the resistance pattern was checked, the Dd2 strain was found to be highly resistant against chloroquine (IC_{50} = 170 nM), pyrimethamine (IC_{50} = 2500 nM), and cycloguanil (IC_{50} = 2200 nM); and moderately resistant against quinine (IC_{50} = 380 nM) and mefloquine (IC_{50} = 57 nM). It was sensitive to halofantrine (IC_{50} = 18 nM), lumefantrine (IC_{50} = 30 nM), artemisinin (IC_{50} = 18 nM), and atovaquone (IC_{50} = 1 nM).
[b] DOXP reductoisomerase inhibition studies were performed under standard assay conditions at compound concentrations of 30 μM.

4. Discussion

Employing a PASP protocol developed by our group, a small series of 3′-amido-3′-deoxy-N^6-(1-naphthylmethyl)adenosines was prepared for biological testing.[13–15] All compounds of this series displayed moderate antimalarial activity against the *P. falciparum* pathogen with IC_{50} values in the low micromolar range. Nonetheless, more striking was the outcome that all 3′-amido-3′-deoxy-N^6-(1-naphthylmethyl)adenosines (**9a–d**) displayed an inhibitory activity against DOXP reductoisomerase, whereas the monosubstituted adenosines of this series had no effect.

Within the series of type IV adenosines, promising diversity fragments established from previous structure-based surveys were introduced into the amino-nucleoside template (**7**). The observed activity on the Dd2 strain of *P. falciparum* is comparable to the activity of type II and III adenosines. In general, the antimalarial activity of disubstituted adenosine derivatives of types II–IV is superior (with IC_{50} values ranging from 1.3 to 26 μM) to the observed antimalarial effect along N^6-monosubstituted adenosines of type I (with IC_{50} values generally in the upper micromolar range).[12]

Considerable inhibitory activity against DOXP reductoisomerase was observed with all 3′,N^6-disubstituted adenosines (**9a–d**). On the contrary, 5′,N^6-disubstituted adenosine derivatives only showed an inhibitory effect in 13 out of 34 cases and N^6-monosubstituted adenosines displayed no inhibitory activity at all. Consequently, disubstitution in 3′- and N^6- or 5′- and N^6-positions seems to be crucial for exhibiting activity against DOXP reductoisomerase.

Compound **9d** revealed activity in the same order of magnitude against the whole parasite compared to the lead structure **1**. While **9d** simultaneously displayed a moderate but significant inhibitory effect on DOXP reductoisomerase (75%), **1** had no effect. This outcome confirms the assumption that a disubstitution of adenosines might be favorable for DOXP reductoisomerase inhibition.

DOXP reductoisomerase inhibition through 3′,N^6-disubstituted adenosine analogues tend to be superior compared to the appropriate 5′,N^6-disubstituted derivatives; for example **9d** (75%) versus **10g** (45%) versus **11b** (48%); and **9c** (41%) versus **10i** (0%). In addition, all 3′,N^6-disubstituted adenosines exhibited DOXP reductoisomerase inhibition whereas only a minor share 5′,N^6-disubstituted analogues showed an inhibitory effect. Nevertheless, this observed tendency might be biased when comparing a small with a large series consisting of four and 34 disubstituted adenosine derivatives, respectively. The correlation between antimalarial activity and DOXP reductoisomerase-inhibition is inconsistent. Several reasons for this observation have to be brought into account. The antimalarial activity is a function of toxicity for the infected erythrocytes and the pathogen and uptake into the pathogen whereas activity in enzyme inhibition tests are less multi-factorial. Enzyme-based measurements were performed as screening at a fixed concentration rather than as careful titration, the rational being to test as many compounds as possible with limited resources. In addition, inter species differences can not be excluded. Enzyme inhibition could only be determined using the *Escherichia coli* derived DOXP reductoisomerase because of the difficulties associated with the handling of the highly analogues *P. falciparum* enzyme. Consequently, further investigations are planned to solidify the assumption made.

The results described above emphasize our earlier suggestion that not only a single molecular target is recognized.[12] Further potential targets include a variety of nucleotide-dependent enzymes, the parasite's nucleoside uptake machinery, and unrelated cell functions.[7,8,11]

The activity obtained is weak compared to established standard drugs and the DOXP reductoisomerase active site inhibitor fosmidomycin. The significance of our results lies in an approach to circumvent the problematic development of active site inhibitors of NADPH utilizing reductoisomerases. Since water has to be excluded from the catalytic process, the active site is narrow and substantial conformational changes of the enzyme upon binding hamper the development of inhibitors starting from the substrate DOXP. Even though active site targeting is the first choice regarding the selectivity achievable, it is obviously rational to complement these efforts by approaches focusing on other binding sites. Therefore our results might serve as proof of principle that blocking of the of the NADPH binding site can be considered as an additional or alternative strategy to interfere with the enzyme function and inhibit the pathogen. While the actual binding to the NADPH binding site was not verified within this work, the combination of both principles in one molecule, active site and cofactor-binding site occupation, has recently been demonstrated to be an elegant and powerful concept to design enzyme inhibitors by the group of Diederich.[26] By combining adenosine and catechol moieties in one molecule, inhibition of the enzyme catechol-*O*-methyltransferase (COMT) was achieved by new potent bisubstrate inhibitors. The bisubstrate binding mode of the compounds was confirmed by X-ray structure analysis.

Experiments to block DOXP reductoisomerase activity by bisubstrate type inhibitors in a similar fashion are under way.

5. Conclusion

The novel series of 3′,N^6-disubstituted adenosines displayed antiplasmodial activity in the low micromolar range as well as DOXP reductoisomerase inhibition. Disubstitution in 3′- and N^6- or alternatively in 5′- and N^6-position seems to be essential for the inhibitory activity on DOXP reductoisomerase, whereas the 3′,N^6-disubstitution is favorable compared to the 5′,N^6-disubstitution. With respect to the modification patterns of types I–IV adenosines, we suggest that not a single molecular target is recognized.

6. Experimental

The structures of all compounds were assigned by NMR spectroscopy. NMR spectra were recorded on a Bruker AMX 400 spectrometer, using tetramethylsilane as internal standard. ^{1}H NMR data are reported based on separated spin-spin signals and protons that were not covered by H_2O- or DMSO-signals, unless otherwise noted. Identity of compounds prepared in mg quantities was evaluated by high-resolution MS; purity was deduced by MPLC/HPLC and from ^{1}H NMR. MPLC purification was performed employing a Büchi 681 pump (flow rate 10 mL, methanol/water gradients) and UV detector (254 nm) with a Merck 310-25 Lobar-LiChroprep^{TM}-RP-18 (40–63 μm) column. HPLC was performed on a Merck Hitachi L-7000 series connected to a diode array detector (methanol/water gradients, flow rate 1 mL/min, UV absorption at 240–261 nm). High-resolution MS data were obtained on a Finnigan MAT 95 XL instrument [ESI, methanol/water (1/1, v/v) infusion at 2 μL/min with polypropylene glycol as reference], MS^n data on a Finnigan MAT 95 XL TRAP instrument [ESI, methanol/water (1/1, v/v) infusion at 2

μL/min]. IR spectra were obtained on a Shimadzu FTIR-8300 instrument. Preparative column chromatography was performed on silica gel 100–200 active, 60 Å, from ICN or Dowex® OH^- (1×2–200) using glass columns (4.5×15 cm). TLC reaction control was performed on Macherey-Nagel Polygram® Sil G/UV_{254} precoated microplates; spots were visualized under UV-illumination at 254 nm.

6.1. 2′,5′-Di-*O*-acetyl-3′-azido-3′-deoxyadenosine (3)

3′-Azido-3′-deoxyadenosine (**2**) (1.0 g, 3.4 mmol) was dissolved in absolute pyridine (12.5 mL). Ac_2O (6.4 mL, 68 mmol) was added and the reaction mixture incubated at room temperature for 1 h. The solvent was evaporated in vacuo. Traces of pyridine were removed by evaporation with toluol three times; traces of Ac_2O by evaporating with H_2O/EtOH (50/50, v/v) two times. The resulting residue was washed with ether yielding **3** as a white and amorphous solid. Yield 61%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.32 (s, 1H, 8H), 8.17 (s, 1H, 2H), 7.36 (bs, 2H, NH_2), 6.18 (d, 1H, 1′H, J = 3.6 Hz), 6.01–5.98 (m, 1H, 3′H), 4.90 (t, 1H, 2′H, J = 6.5 Hz), 4.40–4.36 (m, 1H, 5′CH_2), 4.30–4.25 (m, 1H, 5′CH_2), 4.21–4.17 (m, 1H, 4′H), 2.14 (s, 3H, CH_3, acetyl), 1.98 (s, 3H, CH_3, acetyl). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 169.45, 168.81, 154.80, 151.90, 147.81, 139.05, 85.34, 78.23, 73.48, 61.39, 58.54, 19.37, 19.21. HRESI-MS $[M+H]^+$ calcd 377.1322 found 377.1318.

6.2. 3′-Azido-3′-deoxy-N^6-(1-naphthylmethyl)adenosine (6)

Isoamylnitrite (0.7 g, 6 mmol) was added to a solution of 2′, 5′-di-*O*-acteyl-3′-azido-3′-deoxyadenosine (**3**) (770 mg, 2 mmol) in CCl_4 (12 g, 78 mmol). The reaction mixture was stirred at room temperature for 12 h. As the conversion of **3** to **4** was incomplete, an additional equivalent of isoamylnitrite (230 mg, 2 mmol) was added to the reaction mixture, and again the mixture was stirred at room temperature for 4 h. The solution was evaporated yielding a viscous slurry of **4**. Without further purification, 2′,5′-di-*O*-acetyl-3′-azido-1-(6-chloropurin-9-yl)-β-D-1′,3′-dideoxyribo-furanose (**4**) was dissolved in methanolic ammonia (saturated, 0 °C, 100 mL) and stirred for 1 h. The resulting red solution was then stored for 12 h at 4 °C and subsequently evaporated in vacuo (20 mbar, 20 °C) yielding **5**. Finally, 1-naphthylmethylamine (346 mg, 2.2 mmol) was added to a solution of 3-azido-1-(6-chloropurin-9-yl)-β-D-1,3-dideoxyribofuranose (**5**) in 1-propanol (25 mL). After stirring the reaction mixture at 50 °C for half an h DIPEA (0.17 mL, 1 mmol) was added. The resulting mixture was stirred at 50×C for 4 h and subsequently evaporated in vacuo. Purification by column chromatography with CH_2Cl_2/MeOH gradients as eluent gave **6** as a white solid. Yield 27% (three-step synthesis starting from **4**). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.52 (bs, 1H, N^6H), 8.40 (bs, 1H, 8H), 8.24–8.23 (m, 2H, 2H overlapping naphthyl), 7.96–7.94 (m, 1H, naphthyl), 7.83–7.80 (m, 1H, naphthyl), 7.59–7.52 (m, 2H, naphthyl), 7.44–7.41 (m, 2H, naphthyl), 6.22 (d, 1H, OH, J = 5.3 Hz), 5.93 (d, 1H, 1′H, J = 6.1 Hz), 5.54–5.52 (m, 1H, OH), 5.19 (bs, 2H, CH_2, naphthylmethyl), 5.04–5.01 (m, 1H, 2′H), 4.32 (t, 1H, 3′H, J = 4.1 Hz), 4.00–3.99 (m, 2H, 4′H), 3.71–3.68 (m, 1H, 5′CH_2), 3.61–3.55 (m, 1H, 5′CH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 152.35, 139.88, 133.16, 130.70, 128.41, 127.07, 126.03, 125.62, 125.31, 124.48, 123.25, 87.66, 82.77, 73.77, 61.96, 61.44, 40.92, 39.20. HRESI-MS $[M+H]^+$ calcd 433.1737 found 433.1721.

6.3. 3′-Amino-3′-deoxy-N^6-(1-naphthylmethyl)adenosine (7)

A solution of **6** (225 mg, 0.51 mmol), indium (59 mg, 0.51 mmol) and NH_4Cl (29 mg, 0.56 mmol) in EtOH (10 mL) was refluxed. The reaction was monitored by TLC (EtOAc) and IR. Quantitative conversion was observed after 4 h. Evaporation of the solvent and purification over Dowex® OH^- with MeOH/H_2O gradients yielded **7** as a white, amorphous solid. Yield 62%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.42 (bs, 2H, N^6H overlapping 8H), 8.25–8.22 (m, 2H, naphthyl overlapping 2H), 7.96–7.93 (m, 1H, naphthyl), 7.82–7.80 (m, 1H, naphthyl), 7.59–7.52 (m, 2H, naphthyl), 7.47–7.41 (m, 2H, naphthyl), 5.95 (d, 1H, 1′H, J = 2.8 Hz), 5.77 (bs, 1H, OH), 5.19 (bs, 3H, CH_2 naphthylmethyl overlapping OH), 4.32 (bs, 1H, 2′H), 4.10–4.09 (m, 1H, 3′H), 3.75–3.74 (m, 2H), 3.50–3.48 (m, 1H), 1.64 (bs, 2H, NH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 152.30, 139.24, 133.17, 130.72, 128.40, 127.05, 126.03, 125.61, 125.31, 124.57, 123.27, 89.07, 85.50, 74.67, 60.94, 59.17, 57.09, 52.49, 48.49. HRESI-MS $[M+H]^+$ calcd 407.1832 found 407.1841.

6.4. General procedure A for the synthesis of simple polymer supported acids

To a flask containing 2.0 g of dry 4-sulfamylbenzoylaminomethyl polystyrene with an initial loading level of 1.24 mmol/g as determined by elemental analysis (prepared from very high load aminomethylated polystyrene, purchased from Novabiochem, Switzerland) was added 20 mL of THF. The resin was allowed to swell at room temperature for 2 h. In another flask, 10 mmol of the appropriate acid was dissolved in 10–20 mL dry THF and pre-activated via in situ anhydride formation by adding 780 μL (5 mmol) *N*,*N*-diisopropylcarbodiimide. **CAUTION**: *N*,*N*-diisopropylcarbodiimide may lead to severe allergic reactions, strictly avoid skin contact. After addition of 580 μL Hünig's base (3.4 mmol) and 15 mg (0.12 mmol) 4-(dimethylamino)-pyridine as catalyst, to the swollen resin, the coupling mixture was added. The resulting reaction mixture was agitated at room temperature for 24 h. The resin beads were filtered off and washed exhaustively with DMF (three times 5 mL), dichloromethane (three times 5 mL), and methanol (three times 5 mL). After careful drying the increase in weight was determined. Activation: the sulfonamide linker of 400 mg (approximately 0.4 mmol) of the resins obtained was activated for cleavage by alkylation with 640 μL (9 mmol) bromoacetonitrile, and 340 μL (2 mmol) Hünig's base in 4 mL 1-methylpyrrolidone overnight and washed with dry

dimethylsulfoxide (five times 5 mL) and THF (five times 5 mL).

6.5. General procedure B for the activation of polymer supported acids yielding 8a–d

The sulfonamide linker of the appropriate resins (400 mg) was activated for cleavage by alkylation with bromoacetonitrile (640 μL, 9 mmol) (**CAUTION**: alkylating agent, strictly avoid skin contact), and Hünig's base (340 μL, 2 mmol) in 1-methylpyrrolidone (4 mL) for 12–48 h. The resulting dark brown slurry was washed with dry dimethylsulfoxide (five times 5 mL) and THF (three times 10 mL) leading to ivory colored resin particles.

6.6. General procedure C for the synthesis of compounds 9a–d

Activated polymer-supported acids **8a–d** were transferred to the amino group of 10 μmol of **7** dissolved in 1 mL NMP, by shaking at 55 °C in 4 mL THF. Polymer beads and particulates were removed by filtration; the beads where extracted exhaustively with dry THF and MeOH and the combined THF fractions were evaporated to furnish the target compounds. To remove residual NMP, water (10–20 mL) was added to appropriate **9a–d**, each, and subsequently evaporated in vacuo (20 mbar, 60 °C). This process was repeated four times. Finally, **9a–d** were purified by a single semi-preparative MPLC run under standard conditions. Purity prior to MPLC was estimated using the 100% method, UV detection at 254 nm. All final compounds were obtained as white, amorphous solids.

6.7. 3′-Deoxy-N^6-(1-naphthylmethyl)-3′-(4-phenylbutanamido)adenosine (9a)

Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.45 (bs, 2H, N^6H overlapping 8H), 8.25–8.23 (m, 2H, naphthyl overlapping 2H), 7.96–7.92 (m, 2H, naphthyl overlapping amide), 7.82–7.80 (m, 1H, naphthyl), 7.59–7.52 (m, 2H, naphthyl), 7.47–7.41 (m, 2H, naphthyl), 7.30–7.26 (m, 2H, phenyl), 7.20–7.17 (m, 3H, phenyl), 5.98–5.95 (m, 2H, 1′H overlapping OH), 5.19–5.17 (m, 3H, CH_2 naphthylmethyl overlapping OH), 4.50–4.48 (m, 2H, 2′H overlapping 3′H), 4.02–3.99 (m, 1H, 4′H), 3.73–3.64 (m, 1H, 5′CH_2), 3.56–3.50 (m, 1H, 5′CH_2), 2.58 (t, 2H, CH_2, butyryl, J = 7.7 Hz), 2.19 (t, 2H, CH_2, butyryl, J = 7.2 Hz), 1.84–1.79 (m, 2H, CH_2, butyryl). HRESI-MS $[M+H]^+$ calcd 553.2563 found 553.2574.

6.8. 3′-Deoxy-3′-N^6-(1-naphthylmethyl)-4-[(2-thienyl)butan-amido]adenosine (9b)

Yield 96%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.44 (bs, 2H, N^6H overlapping 8H), 8.25–8.23 (m, 2H, naphthyl overlapping 2H), 7.97–7.93 (m, 2H, naphthyl overlapping amide), 7.82–7.80 (m, 1H, naphthyl), 7.59–7.52 (m, 2H, naphthyl), 7.47–7.41 (m, 2H, naphthyl), 7.31–7.30 (m, 1H, thienyl), 6.95–6.93 (m, 1H, thienyl), 6.85–6.84 (m, 1H, thienyl), 5.98–5.97 (m, 2H, 1′H overlapping OH), 5.21–5.17 (m, 3H, CH_2 naphthylmethyl overlapping OH), 4.50–4.47 (m, 2H, 2′H overlapping 3′H), 4.03–3.99 (m, 1H, 4′H), 3.73–3.68 (m, 1H, 5′CH_2), 3.57–3.49 (m, 1H, 5′CH_2), 2.80 (t, 2H, CH_2, butyryl, J = 7.5 Hz), 2.69 (t, 2H, CH_2, butyryl, J = 7.5 Hz), 1.88–1.81 (m, 2H, CH_2, butyryl). HRESI-MS $[M+H]^+$ calcd 559.2127 found 559.2129.

6.9. 3′-[4-(2,4-Dichlorophenyloxy)butanamido]-3′-deoxy-N^6-(1-naphthylmethyl)adenosine (9c)

Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.45 (bs, 2H, N^6H overlapping 8H), 8.25–8.23 (m, 2H, naphthyl overlapping 2H), 8.04 (d, 1H, NH, amide, J = 7.1 Hz), 7.96–7.94 (m, 1H, naphthyl), 7.82–7.80 (m, 1H, naphthyl), 7.59–7.52 (m, 3H, 2H naphthyl overlapping 1H phenyloxy), 7.47–7.41 (m, 2H, naphthyl), 7.37–7.34 (dd, 1H, phenyloxy, J = 2,5 Hz, 8,9 Hz), 7.17 (d, 1H, phenyloxy, J = 9.2 Hz), 5.98 (bs, 2H, 1′H overlapping OH), 5.21–5.18 (m, 3H, CH_2 naphthylmethyl over-lapping OH), 4.51–4.49 (m, 2H, 2′H overlapping 3′H), 4.08 (t, 2H, CH_2, butyryl, J = 6.4 Hz), 4.03–4.02 (m, 1H, 4′H), 3.73–3.69 (m, 1H, 5′CH_2), 3.56–3.51 (m, 1H, 5′CH_2), 2.37 (t, 2H, CH_2, butyryl, J = 7.4 Hz), 2.01–1.94 (m, 2H, CH_2, butyryl). HRESI-MS $[M+H]^+$ calcd 637.1734 found 637.1749.

6.10. 3′-Deoxy-3′-[4-(3-indolyl)butanamido]-N^6-(1-naphthylmethyl)adenosine (9d)

Yield 70%. ^{1}H NMR (400 MHz, DMSO-d_6) = δ (ppm) 10.74 (bs, 1H, NH, indolyl), 8.45 (bs, 2H, N^6H overlapping 8H), 8.25–8.23 (m, 2H, naphthyl overlapping 2H), 7.96–7.90 (m, 2H, naphthyl over-lapping amide), 7.82–7.80 (m, 1H, naphthyl), 7.59–7.42 (m, 5H, 4H naphthyl overlapping 1H indolyl), 7.33 (d, 1H, indolyl, J = 7.6 Hz), 7.10 (bs, 1H, indolyl), 7.07–7.03 (m, 1H, indolyl), 6.99–6.94 (m, 1H, indolyl), 5.98–5.94 (m, 2H, 1′H overlapping OH), 5.18 (bs, 3H, CH_2 naphthylmethyl overlapping OH), 4.50 (bs, 2H, 2′H overlapping 3′H), 4.03–4.00 (m, 1H, 4′H), 3.74–3.69 (m, 1H, 5′CH_2), 3.56–3.51 (m, 1H, 5′CH_2), 2.69 (t, 2H, CH_2, butyryl, J = 7.6 Hz), 2.28–2.22 (m, 2H, CH_2, butyryl), 1.92–1.86 (m, 2H, CH_2, butyryl). HRESI-MS $[M+H]^+$ calcd 592.2672 found 592.2670.

6.11. In vitro measurement of *P. falciparum* growth inhibition[23–25]

The *P. falciparum* strain Dd2 was cultivated by a modification of the method described by Trager and Jensen. The culture medium consisted of RPMI 1640 supplemented with 10% human type 0^+ serum and 25 mM HEPES. Human type 0^+ erythrocytes served as host cells. The cultures were kept at 37 °C in an atmosphere of 5% O_2, 3% CO_2, and 92% N_2. Testing of compounds was carried out in 96-well microtiter plates. The compounds were dissolved in DMSO (10 mM) and prediluted in complete culture medium. Infected erythrocytes (200 μL per well, with 2% hematocrit and 0.4% parasitemia) were incubated in duplicate with a serial dilution of the compounds for 48 h. After the addition of 0.8 μCi [^{3}H]-hypoxanthine in 50 μL medium per well, the plates were further incubated for 24 h. Cells were collected on glass fiber filters with a cell har-

vester (Micromate 196, Packard) and incorporated radioactivity measured using a β-counter (Matrix 9600, Packard).

6.12. Measurement of DOXP reductoisomerase inhibition

For the recombinant production of DOXP reductoisomerase the *dxr* gene was PCR amplified from genomic DNA of the *E. coli* strain HB101 using the primers ecoldxrfor (gcggatccatgaagcaactcaccattctg) and ecoldxrrev (ccggaagctttcagcttgcgagacgcatca). The PCR product was cloned into the pCR2.1 vector (Invitrogen) and further subcloned into the expression vector pQE9 (Qiagen) using the BamH I and Hind III restriction sites. The resulting histidine hexamer fusion protein was purified to approximately 99% homogenity by metal affinity, anion exchange, and gel permeation chromatography. The enzyme inhibition assay was performed in a reaction mixture containing 100 mM Tris HCl (pH 7.5), 1 mM $MnCl_2$, 1 mM DOXP, 0.3 mM NADPH, and 1 μg/mL DOXP reductoisomerase in a total volume of 0.2 mL on a 96 well microtiter plate. The test compounds were dissolved in DMSO and added to the reaction mixture yielding a final concentration of 30 μM. The reaction was started by addition of DOXP. The conversion of NADPH was monitored by measuring the decrease of absorption at 340 nm using a SpectraMax 340PC microplate reader (Molecular Devices, Ismaning).

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